ABSTRACT

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Title of Diploma thesis: Analysis of lipids in epidermis with filaggrin knock-down

Filaggrin (FLG) is crucial for correct development of epidermis and for its function as a skin barrier. Mutation in the FLG gene and loss of its function is associated with diseases such as ichthyosis vulgaris and atopic dermatitis. The aim of the study was to evaluate the impact of FLG knock-down on the composition of intercellular lipids in the stratum corneum (SC). We used in vitro skin constructs with reduced (FLG-) and normal gene expression (FLG+) for the analysis. It enabled us to study separately the influence of FLG on the composition and organization of SC lipids.

FLG is a protein which is degraded to metabolites which cause an acidification of SC. pH is important for optimal activity of enzymes which are pivotal for formation of ceramides from their precursors. Therefore we assumed lower ceramide content in skin barrier when FLG is reduced. Our assumption was supported with findings in patients with atopic dermatitis. However our HPTLC analysis showed that the amount of lipids is similar in both FLG- and FLG+ skin construct. The only statistically significant difference was the nearly twofold higher content of free fatty acids in FLG- construct.

Based on this finding, pH of SC and an activity of secretory phospholipase (PLA_2) were evaluated. SC pH was optimal and increased PLA_2 activity was confirmed. This means, that conversion of phospholipids into fatty acids by PLA_2 is another mechanism how to acidify SC. Nevertheless, the increased level of free fatty acid causes decreased skin lipid order. Disorder and higher mobility of hydrophobic chains has a negative effect on skin barrier since it becomes more permeable for lipophilic substances.

In conclusion, we found a feedback mechanism between FLG and fatty acid-based acidification pathways in the skin. The lower ceramide levels previously described in atopic dermatitis are probably caused by mechanisms independent of FLG.